coupled within an active RecBCD enzyme. (supported by NIH GM045948 to TML).

1326-Pos Board B236
Towards In Vivo Tracking of the E. coli Replisome Utilizing Single-Molecule Fluorescence Microscopy
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The bacterium Escherichia coli replicates its circular chromosome in a bi-directional manner, with individual replisomes simultaneously synthesizing DNA. Replication is an extremely crucial process where even the slightest mistake can have dire consequences for the cell. Much of our current knowledge concerning the dynamics of the replisome has been obtained from in vitro experiments not performed on the single-molecule level. The natural environment of the cell is considerably different than that of in vitro solutions. We for example do not yet have sufficient detail on the exact copy numbers of the different molecules in a cell during cell division. These differences can have a substantial influence on how certain proteins in a cell function. Single-molecule techniques provide one with the added insight into events that find place in the cell during replication that are not apparent in ensemble averaging. It is thus essential that more quantitative in vivo single-molecule research be conducted before we can fully understand the details of DNA replication as it occurs in a living cell. In our research we aim to use single-molecule fluorescence microscopy to track individual replisomes, and investigate their dynamics during the process of DNA replication in living bacterial cells. Here we describe the microscopy and genetic techniques involved in such experiments, as well as what our research outlook entails.

1327-Pos Board B237
Helicase-Primase Interactions and their Structural Studies in Helicobacter Pylori
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In eubacterial DNA replication initiation complex, two proteins of prime foci are DnaB helicase, a homohexameric motor protein which unwinds duplex DNA, and DnaG primase, a DNA dependent RNA polymerase which synthesizes oligonucleotide primers for DNA replication.
In our study we have found the H. pylori DnaB helicase activity being stimulated by H. pylori DnaG primase, suggesting the presence of helicase-primase cohort at replication fork. We have reported the crystal structure of NTD of HpDnaB helicase at 2.2 Å resolution. The structural details of NTD besides SPR studies suggest that the linker region between NTD and C-terminal helicase domain plays a vital role in physiological assembly of NTD dimers, and strong interaction with C-terminal domain (CTD) of primase. The sequence analysis of the linker region reveals that they should form four helix bundles. The surface charge distribution on the primase binding surface of NTD's of various helicases shows that the DnaB-DnaG interaction and stability of the complex appears to be charge dependent.
Experimental phasing (SAD) was used to solve the crystal structure of HpDnaG primase CTD from 1.7 Å diffraction data. The structure is helical in nature like the other two eubacterial DnaG primase CTD structures of Bacillus stearothermophilus and Escherichia coli. Structural comparison suggests that different orientation of helical hairpin in the DnaG primase CTD may play an important role in the regulation of primase activity.

1328-Pos Board B238
Towards the Kinetic Mechanism of S. cerevisiae MutLλ DNA Mismatch Repair Protein
Anushi Sharma, Manju Hingorani.
Mismatch repair (MMR) is essential for correcting base-pairing errors in DNA. The MMR mechanism is most well understood in *E. coli*, where MutS initially binds a mismatched base-pair and recruits MutL, which in turn activates MutH, an endonuclease that nicks DNA to initiate excision and resynthesis. In eukaryotes, the MutL homologue itself has endonuclease activity, and its role and mechanism of action in MMR are under active investigation. Since MutL is a critical connecting link between mismatch recognition and initiation of repair, we are interested in understanding its mechanism of action. To this end, we are currently characterizing *S. cerevisiae* MutLα over-produced in *E. coli*, with the aid of chaperonins, and purified by 3-column ion exchange chromatograpy; initial analysis has demonstrated that the recombinant protein is an active endonuclease. In addition we are developing assays for transient kinetic analysis of MutLα. Specifically, we are using a PCR-based strategy to synthesize large quantities of a 200 bp DNA substrate that contains a mismatched base-pair, with an adjacent 2-Aminopurine to detect MutSα binding, is of sufficient length to support MutLα binding, and has a pre-existing nick to stimulate MutLα endonuclease activity. We plan stopped-flow and quench-flow DNA binding, ATPase, and endonuclease experiments with these reagents to directly observe the events in the pathway between mismatch recognition and initiation of DNA repair.

1329-Pos Board B239
Single Molecule Studies on the RNA Polymerase QDE-1 by Optical Tweezers
Gabija Ziedaite, Anders E. Wallin, Heikki Ojala, Kalle Hanhijärvi,
Antti Aalto, Edward Hæggström, Dennis H. Bamford.
Cellular regulatory mechanisms which rely on small dsRNA molecules (RNA silencing) are major (new) discoveries in biology.
In many eukaryotic organisms silencing is achieved post-transcriptionally through pathways where dsRNA is synthesized by RNA-dependent RNA polymerases (RdRPs) and processed into 21-25 nucleotide long RNA molecules.
Here we study QDE-1, an RdRP involved in the quelling (RNA silencing) pathway of Neurospora crassa. This filamentous fungus displays noticeable genomic stability, which has been attributed to quelling and other silencing mechanisms. Recently it was shown that QDE-1 is more active as a DNA-dependent RNA polymerase (DdRP) than as an RdRp and that it is also involved in DNA damage response (1,2). Recombinant QDE-1 displays five different enzymatic activities in vitro (3). The structural and biochemical data on this enzyme is extensive (1-5).
We are interested on the basic biophysical parameters of QDE-1 action. The approaches taken rely on single molecule assays using high resolution optical tweezers, combined with fluorescence imaging. An optically levitated 'dumbbell' assay is used: the nucleic acid (NA) construct features one or two biotinylated ends that tether two different microspheres. The protein of interest is attached to the microsphere or directly to the NA tether. Since in our double-trap instrument one trap is stable whereas the other mobile, we can manipulate the tethers, detect changes in tether length and stiffness, and apply different forces and simultaneously observe the mobility of the fluorescently labelled template or protein.
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1330-Pos Board B240
Structure-Function Analysis of the S. Cerevisiae PCNA Clamp-An Essential Protein in DNA Metabolism
Yayan Zhou, Manju Hingorani.
Circular clamp proteins enable processive DNA replication by tethering polymerases to the primer-template during DNA replication. Clamps also bind to and coordinate the functions of many other proteins on DNA, and are therefore essential for a variety of DNA metabolic processes. Clamps are loaded onto DNA in an ATP-fueled reaction by multi-subunit AAA+ protein complexes known as Clamp Loaders. The mechanism of action of these proteins is under active investigation, given their important role in genomic DNA replication, repair and recombination. According to our current kinetic model of the *S. cerevisiae* RFC clamp loader, ATP binding to RFC activates the clamp loader, allowing it to bind and open the PCNA clamp for entry of primer-template DNA. DNA binding triggers ATP hydrolysis, PCNA closure around DNA and release of the PCNA-DNA complex from RFC. At present, we are investigating dynamic interactions between RFC, PCNA and DNA associated with key events in the reaction as described above. In particular, we are exploring the possibility that interactions between positively charged residues on the inside of the clamp and the DNA play an important role in triggering clamp closure around DNA and catalytic turnover. In order to test this hypothesis, we have prepared several PCNA mutants in which individual conserved amino acids have been substituted with Alanine. Data from transient kinetic analysis of the PCNA opening/closing, DNA binding/release, and ATP binding/hydrolysis steps in the reaction indicate that even a single positively charged amino acid in PCNA can make a substantive contribution to the reaction mechanism. These findings will help us to understand how contact between PCNA and DNA influences both PCNA assembly and function on DNA.